Department of HEALTH, EDUCATION, AND WELFARE • Public Health Service
National Institutes of Health • Bethesda 14, Md.

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
NATIONAL CANCER INSTITUTE
NATIONAL INSTITUTE OF DENTAL RESEARCH
NATIONAL HEART INSTITUTE
NATIONAL INSTITUTE OF MENTAL HEALTH
NATIONAL MICROBIOLOGICAL INSTITUTE
NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS
THE CLINICAL CENTER
DIVISION OF RESEARCH GRANTS

Hinshaw

January 25, 1957

Drs. Esther M. and Joshua Lederberg
University of Wisconsin
College of Agriculture
Agricultural Hall
Madison 6, Wisconsin

Dear Drs. Lederberg:

Thank you very much for your letter of January 7 and reprints of your papers.

I am sorry for delay of the mapping of enzymes and quantitative data. We have moved from the 9th floor to the 8th floor at the end of the last year and are just about settling down. I think I can carry out the mapping rather soon.

As to the inhibition study of growth of Gal-1-P transferase-less mutants by galactose, I'll give you the following conditions under which we observed the phenomena.

Cells of W3096 were grown in galactose complete media (10 gm. of casein digests, 5 gm. of yeast extract, 3 gm. of K_2HPO_4, 1 gm. of $K H_2PO_4$ and 5 gm. of galactose per liter) at 37° C for 12 to 16 hours. The cells are washed with 0.9% NaCl or water twice in order to remove organic substrate.

The washed cells were resuspended in enough water to give optical density 0.30 upon 1 to 25 dilution at 660 mμ in Beckman DU with Klett tube attachment. (that is, the O.D. of the cell suspension itself about 7.5). 0.2 ml. of the above cell suspension was inoculated into 5 ml. of synthetic medium (13.6 gm. of KH_2PO_4; 2 gm. of $(NH_4)_2SO_4$, 0.2 gm. of $MgSO_4 \cdot 7H_2O$, 0.01 gm. of $CaCl_2$, 0.0005 gm. of $Fe SO_4 \cdot 7 H_2O$ and KOH to bring the pH to 7.2 per 1 l. of medium). To one tube 0.05 ml. of 20% glucose was added and to the other 0.05 ml. of 20% glucose and 0.05 ml. of 20% galactose was added. The inoculated tubes are incubated at 37°.

After 18 hours incubation, the tube with glucose alone showed O.D. 0.20 and the one with glucose and galactose 0.06.

The above is an exact copy of protocol of one of the experiments, but the similar effect can be observed with larger culture too. Two points are important: 1) the inoculum must be free from organic substance and small, 2) the media for observing the inhibition by galactose must be synthetic.

By the above technique, you can screen gal-1-p uridyl transferase-less mutants. Not appreciable inhibition by galactose was observed with wild type and galactokinaseless mutants.

I also very much appreciated your kind consideration to give me a chance to work at Madison. I am very interested in this possibility but before Christmas I was informed about an opening at Osamu Hayaishi's section and have more or less committed myself to take up this position in May. I am sorry to miss such a nice opportunity to learn about phage genetics from you. If by some reason I would not be able to be appointed as a visiting scientist here, I would like to consider again this possibility. I think my plans would become cleared by early summer.

I hope I can write to you soon the results on W3264 and W3265.

Sincerely yours,

Kiyoshi Kurahashi

Kiyoshi Kurahashi

KK:lh